



Analysis of Lipophilic Constituents Related to Heartwood Formation in Young *Swietenia mahagoni* (L.) Jacq Trees

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ABSTRACT

Swietenia mahagoni is one of the commercial timbers in Indonesia. Mahogany heartwood is an important characteristic as it relates to the natural durability and aesthetics of the wood. Lipophilic extractives are known to be involved in the heartwood formation process. Therefore, this study aims to determine the lipophilic compounds associated with heartwood formation. The *n*-hexane extract from sapwood and heartwood samples (1 to 5 years) was analyzed by gas chromatography-mass spectrometry. The results showed that the content of *n*-hexane extract ranged from 0.76% to 2.45% based on dry wood. The main group of compounds identified in the lipophilic fraction consisted of sterols (β -sitosterol, stigmasterol, campasterol, and cyclolaudenol), fatty acids (palmitic, oleic, linoleic, and stearic acid), and hydrocarbons (pentadecane, 1-octadecane, hexadecane, cyclotetracosane, cycloeicosane, and cyclooctacosane) after heartwood formation. In addition, the hydrocarbon fraction was the largest, followed by sterols, fatty acids, and 1-heneicosanol. In the radial variation, the distribution of fatty acids was greater in the sapwood than in the heartwood (4-year-old). However, the reverse pattern was found at the age of 5 years. The lipophilic fraction was generally more abundant in the heartwood compared to the sapwood, especially at 5 years of age, with much higher levels than when the heartwood was forming (4 years). These findings show that when the heartwood formation begins, the lipid composition was not fully metabolized at the beginning of heartwood formation compared to 5-year-old trees.

Keywords: lipophilic extractive, heartwood formation, sterols, fatty acids, *Swietenia mahagoni*

1. INTRODUCTION

Indonesia is a tropical country and is known as the third largest mega-biodiversity country in the world (Savero *et al.*, 2020). At least, 4,000 tree species exist in the country's tropical rainforests (Martawijaya *et al.*, 2005). One of wood species that is quite popular in

Indonesia is mahogany (*Swietenia mahagoni*). In Indonesia, it is a popular raw material for veneer decorations, plywood, and furniture (Martawijaya *et al.*, 2005). The heartwood of *S. mahagoni* is resistant to decay and insect attack and performs better on the global market than *Swietenia macrophylla* and *Swietenia humilis* (Sarhean *et al.*, 2015). Febrianto *et al.* (2015) stated that

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mahogany wood was included in durability class II. Arinana *et al.* (2022) reported that the wood of *S. mahagoni* was the last of the six species of wood preferred by the subterranean termites. Cahyono *et al.* (2020) also found that laminates made from *S. mahagoni* wood veneers were the most resistant to drywood termite attack. Furthermore, Yanti *et al.* (2019) obtained that the modulus of elasticity and modulus of rupture values of oriented strand board increased after being coated with wood veneer from *S. mahagoni*. Tannin resorcinol formaldehyde prepared from mahogany bark extract was also reported to be more environmentally friendly due to low formaldehyde emissions (Trisatya *et al.*, 2023). The mahogany heartwood is the most marketable component for which international timber markets expect premium payments (Wadsworth and González, 2008). The presence of heartwood is an important factor in determining the grade and its potential use (Seta *et al.*, 2023). The colour of mahogany heartwood is reddish or pink, and darkens with age to dark red or brown. Genetic factors are thought to be the main reason for the emergence of variation in heartwood colour. However, not all heartwood with similar genes will show the same colour (Won *et al.*, 2017). Genetic factors are also thought to control growth characteristics (tree height, diameter and volume) and basic density (Fadwati *et al.*, 2023).

According to the International Association of Wood Anatomists (IAWA, 1964), heartwood is wood that no longer contains living cells in growing trees where reserve materials such as starch have been converted into heartwood substances. Meanwhile, sapwood is wood that contains living cells in living trees and has reserve materials such as starch (Anonymous, 1957). Parenchyma cells in the transition zone between sapwood and heartwood undergo metabolic changes prior to cell death (Piqueras *et al.*, 2020). This results in increased synthesis of secondary metabolites called extractives. Extractives, a naturally occurring minor component of wood, are essential in protecting wood from decay by wood-

destroying organisms (Darmawan *et al.*, 2018). Previous studies have also reported the potential biological activity of extracts soluble in various polar solvents (i.e. methanol, ethanol, and distilled water) from several species such as *Azadirachta excelsa* seeds and *Guibourtia tessmanii* bark for anti-termite (Adfa *et al.*, 2023; Ella Nkogo *et al.*, 2022), herbal plants of *Phellodendri cortex*, *Magnoliae cortex* and some plants for antifungi (Na and Kim, 2022; Yoon and Kim, 2023), 12 herbal plants for antimicrobial resistance (Ham and Kim, 2022), *Quercus gilva* and *Citrus × natsudaidai* for antioxidant activity (Huh *et al.*, 2022; Yang *et al.*, 2022a) and the *Lauraceae* family for anti-asthmatic activity (Yang *et al.*, 2022b).

Several basic properties play an important role in the utilization of wood as a raw material, one of them is information about the characteristics of the heartwood (Iswanto *et al.*, 2021). The process of heartwood formation is known to be associated with storage depletion, parenchyma cell death, and increased extractives (Hillis, 1987). The depletion of reserves, starch, non-structural carbohydrates (NSC) and triacylglycerol is a form of parenchyma cell disintegration. In the outer sapwood, which contains many starch granules, starch gradually decreases to the transition zone. Starch in the middle sapwood is hydrolysed by phosphatases to sugar phosphates and thus becomes a precursor for the synthesis of extractives or lipids. Small lipophilic droplets were found in the axial and ray parenchyma of the outer sapwood. These values were significantly increased in the middle and inner sapwood as well as in the transition zone. Lipids and carbohydrates in these tissues are thought to provide the carbon skeleton for extractive synthesis in heartwood (Kampe and Magel, 2013). Heartwood is known to be free of storage material. Therefore, during heartwood formation, starch and lipid are degraded and play an important role as carbon skeletons for extractive phenolic biosynthesis and senescence of ray parenchyma cells (Hillis, 1987; Magel, 2000; Song *et al.*, 2011).

It is well known that lipids such as fatty acids, resin acids and sterols have been analysed to find out their involvement in the process of heartwood formation. In some previous studies, it was known that the amount of fatty acids and sterol composition increased from sapwood to heartwood due to the heartwood formation process in some species such as *Pinus radiata* and *Pinus sylvestris* (Hemingway and Hillis, 1971; Hillis, 1972; Höll and Goller, 1982; Kampe and Magel, 2013; Saranpää and Nyberg, 1987). In addition, the extractive composition of heartwood was greater than that of sapwood (Hillis, 1971; Miranda *et al.*, 2006). The accumulation of extractives in the heartwood is a key feature of heartwood formation (Hillis, 1971).

Most sterols in plants are found in cellular organelles (Grunwald, 1970) and in the plasmalemma (Hartmann *et al.*, 1975). It has been suggested that the interaction of sterols with phospholipids stabilises the membrane. This will be control permeability (Grunwald, 1980). Sterol levels were found to be higher in heartwood than in sapwood (Höll and Goller, 1982). To the best of our knowledge, there is no study of the lipophilic extractives in *S. mahagoni* wood associated with heartwood formation. Therefore, the aim of this study was to identify the lipophilic extractives and their proportion in relation to heartwood formation in *S. mahagoni*.

2. MATERIALS and METHODS

2.1. Sample collection

For the preliminary studies, the 4-year-old trees had been collected in December 2018, while the 2- and 3-year-old trees had been obtained in October 2019. In the meantime, the 1- and 5-year-old trees were felled in October 2020. Three different trees from the lower part (5% of the total merchantable height) were sampled for each tree age. Young tree samples (1–5 years old) were harvested from the Perum Perhutani plantation in

Temanggung Regency, Central Java, Indonesia (7°14' S, 110°2' E) during the rainy season. Table 1 describes the tree age, diameter at breast height and total merchantable height.

At one to three years of age, visual inspection of the mahogany trunk in cross section showed no signs of discolouration. Meanwhile, at the age of four years a colour change occurred with a distinct salmon pink to red colour in comparison to the light-coloured sapwood. The area of heartwood formation was then identified at this colour change. Samples from the 1- to 3-year-old trees were taken from the sapwood only as they heartwood areas were not present. Subsequently, the disc samples were stored at room temperature and air-dried. Disc samples in the cardinal direction were then drilled to sample the wood. Samples of sapwood (0.5 cm from the bark-sapwood boundary) and heartwood (1.0 cm from the sapwood-heartwood boundary) were collected for chemical analysis. The drilled wood was then separately ground into powder and sieved (through a 1 mm sieve) for chemical analysis.

2.2. Extraction

Powders of 0.8 g (1-year-old plants) and 1.5 g (2- to 5-year-old plants) were extracted with *n*-hexane (60°C

Table 1. Description of tree samples from three individual young *Swietenia mahagoni* trees

No	Age	Average of DBH (cm)	Average of total merchantable height (m)
1	1	1.23 ± 0.21 ¹⁾	0.53 ± 0.11
2	2	2.03 ± 0.35	1.85 ± 0.10
3	3	4.57 ± 0.27	4.06 ± 0.29
4	4	5.92 ± 1.25	4.93 ± 1.01
5	5	8.72 ± 0.83	6.95 ± 1.54

¹⁾ Diameter is taken at the level height 10%.
DBH: diameter at breast height.

for 6 h) in a Soxhlet apparatus. There were 8 cycles per hour during the extraction process. The solvent was dried by evaporation and the weight of the extract is expressed as a percentage of dry wood.

2.3. Gas chromatography-mass spectrometry analyses

The lipophilic constituent was identified by Gas Chromatography-Mass Spectrometry (GC-MS-QP 2010, Shimadzu, Kyoto, Japan) under the following conditions: RTX-5MS capillary column (30 m × 0.25 mm I.D. and 0.25 μm; GL Sciences, Tokyo, Japan); carrier gas He; injector temperature 200°C; oven initial temperature from 70°C (2 min) to 290°C at 5°C/min; detection temperature 285°C; acquisition mass ranged from 50 to 666 amu. The method used trimethylchlorosilane (TMCS) derivatization and the samples were derivatized before injection according to the method described by Moldeveanu and David (2018) with modifications. Furthermore, a mixture of 100 μL N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) and 1% TMCS was

added to 2 mg of each dry *n*-hexane extract diluted in 100 μL pyridine. Before injecting 1 μL (1 mg/mL) into the GC-MS, 1 mL of *n*-hexane solution was added to the mixture and sonicated at room temperature. The compounds were identified by comparing experimental GC-MS data with authentic compounds, the NIST MS library (NIST, 2011), and research literatures (Arisandi *et al.*, 2020; del Río *et al.*, 2011; Domingues *et al.*, 2010; Ferreira *et al.*, 2018; Freire *et al.*, 2002a, 2002b, 2006a, 2006b; Gutiérrez *et al.*, 1999, 2006; Hafizoğlu *et al.*, 2002; Silvério *et al.*, 2007).

2.4. Quantification of lipophilic constituents

β -sitosterol was used to prepare a calibration curve (Fig. 1) for the quantification of sterol fractions with a concentration range of 0.2, 0.1, 0.05, and 0.00625 μg/μL ($R^2 = 0.995$). For the quantification of fatty acids, palmitic acid was used to obtain the calibration curves (Fig. 2) at the range of 0.3, 0.1, 0.05, 0.01, 0.0025, and 0.000625 μg/μL ($R^2 = 0.999$). The respective response factors were calculated as the average of three GC-MS

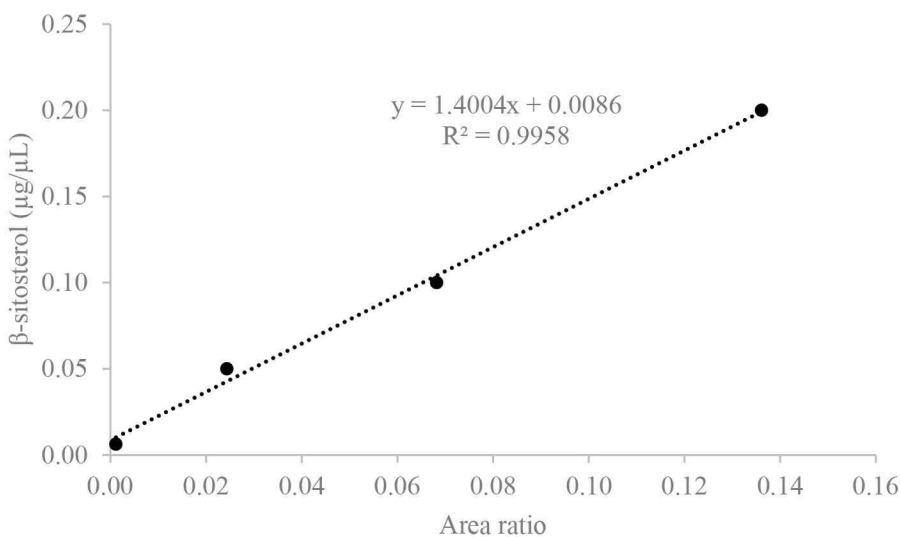


Fig. 1. Calibration curve of β -sitosterol.

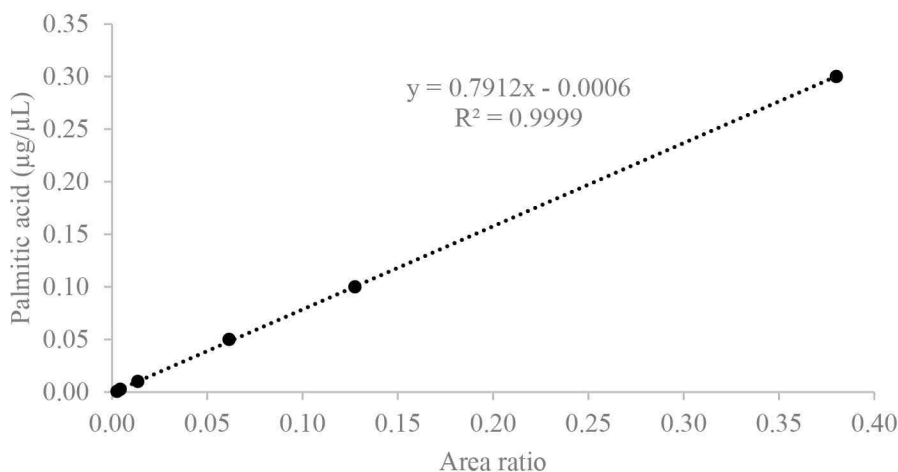


Fig. 2. Calibration curve of palmitic acid.

runs, and other constituents were compared to internal standards, such as veratric acid.

2.5. Chemicals

Standard compounds, including, dodecanoic, myristic, pentadecanoic, palmitic, oleic, linoleic, stearic, arachidic, docosanoic, and tetracosanoic acid were obtained from Shanghai Chemical Industry (Shanghai, China). Meanwhile, veratric acid and β -sitosterol were purchased from Kanto Chemical (Tokyo, Japan).

3. RESULTS and DISCUSSION

3.1. Extractive content and lipophilic identification

The total extractive content of *n*-hexane extract was from 0.76% to 2.45% based on dried wood (Table 2). Non-polar solvents such as *n*-hexane, dichloromethane, and chloroform, are commonly used for the analysis of lipophilic compounds (Arisandi *et al.*, 2020; Freire *et al.*, 2006a, 2006b; Gominho *et al.*, 2020; Gutiérrez *et*

al., 1999; Silvério *et al.*, 2007). A total of four lipophilic groups were detected in the species examined, namely hydrocarbons, fatty acids, sterols, and others (Table 2, Fig. 3).

The abundant hydrocarbon compounds were penta-decane, 1-octadecene, hexadecane, cyclotetracosane, cycloicosane, and cyclooctacosane. Meanwhile, the few compounds were cetene, undecane, 3-methylene-, tridecane, 3-methylene-, heptadecane, and heneicosane. In other species such as *S. macrophylla* in the bark part, Arisandi *et al.* (2019) reported that 1-octadecane, penta-decane, and cyclooctacosane were the most abundant, while heptadecane was detected as a minor component. In addition, 1-tridecane and 1-octadecane were recorded in *Eucalyptus globulus* (Gominho *et al.*, 2020). The hydrocarbon fraction in this study was indeed the largest. It belongs to the lipophilic group with a large amount in the wood, as reported by some studies in *E. globulus* species (Gutiérrez *et al.*, 1999; Swan, 1967).

Fatty acid groups, namely, dodecanoic, myristic, pentadecanoic, palmitic, oleic, linoleic, stearic, arachidic, docosanoic, and tetracosanoic acids were detected in the *S. mahogany* wood. Other studies such as in *E. globulus*,

Table 2. Lipophilic constituents of *n*-hexane extract from *Swietenia mahagoni* wood (% based on dried extract)

Compounds and extractives	Formula	1 year	2 year	3 year	4 year		5 year	
		Sapwood	Sapwood	Sapwood	Sapwood	Heartwood	Sapwood	Heartwood
Extractive		0.76 (0.13)	1.27 (0.10)	1.54 (0.52)	1.67 (0.37)	1.83 (0.56)	1.50 (0.55)	2.45 (0.16)
Hydrocarbons		12.70	16.00	3.91	18.60	23.60	9.51	19.10
Undecane, 3-methylene- ^{**}	C ₁₂ H ₂₆	0.00	0.00	1.40 (0.52)	1.11 (0.57)	0.59 (0.31)	0.00	1.46 (0.73)
Tridecane, 3-methylene- ^{**}	C ₁₄ H ₂₈	0.00	0.00	1.33 (0.60)	0.23 (0.28)	0.30 (0.09)	0.00	0.31 (0.13)
Pentadecane ^{**}	C ₁₅ H ₃₀	1.26 (0.58)	1.27 (0.33)	0.00	5.39 (2.65)	2.17 (0.46)	1.17 (1.39)	1.88 (0.43)
Cetene ^{**}	C ₁₆ H ₃₂	0.00	0.00	0.38 (0.12)	0.00	3.19 (1.06)	1.59 (1.18)	2.38 (1.10)
Hexadecane ^{**}	C ₁₆ H ₃₄	2.75 (2.44)	0.81 (0.14)	0.00	4.66 (2.52)	0.53 (0.36)	0.00	1.62 (0.20)
Heptadecane ^{**}	C ₁₇ H ₃₆	0.00	0.61 (0.07)	0.72 (0.30)	3.71 (2.26)	0.00	0.00	0.00
1-Octadecene ^{**}	C ₁₈ H ₃₆	2.12 (1.49)	4.66 (0.73)	0.08 (0.03)	0.41 (0.17)	3.02 (1.19)	2.45 (1.96)	2.39 (0.80)
Cycloeicosane ^{**}	C ₁₈ H ₃₆ O ₂	2.78 (2.48)	2.96 (0.73)	0.00	0.00	6.40 (2.47)	2.90 (1.49)	2.28 (1.03)
Heneicosane [*]	C ₂₁ H ₄₄	1.69 (1.46)	0.45 (0.41)	0.00	2.95 (1.57)	0.00	0.00	0.40 (0.16)
Cyclotetracosane [*]	C ₂₄ H ₄₈	0.00	4.30 (0.52)	0.00	0.12 (0.10)	5.10 (2.01)	0.00	5.39 (0.93)
Cyclooctacosane ^{**}	C ₂₈ H ₅₆	2.14 (1.87)	0.98 (0.37)	0.00	0.00	2.30 (0.68)	1.40 (1.03)	0.98 (0.20)
Fatty acids		8.80	9.31	7.66	16.7	13.7	8.89	16.8
Dodecanoic acid [*]	C ₁₂ H ₂₄ O ₂	0.24 (0.06)	0.00	0.00	0.28 (0.11)	0.00	0.24 (0.31)	0.00
Myristic acid [*]	C ₁₄ H ₂₈ O ₂	0.59 (0.24)	0.00	0.00	0.17 (0.04)	0.00	0.00	0.00
Pentadecanoic acid [*]	C ₁₅ H ₃₀ O ₂	0.37 (0.12)	0.00	0.00	0.15 (0.04)	0.11 (0.07)	0.00	2.04 (1.30)
Palmitic acid [*]	C ₁₆ H ₃₂ O ₂	3.99 (0.66)	5.29 (0.87)	5.15 (2.40)	6.08 (1.32)	6.27 (0.85)	3.67 (1.76)	6.06 (2.93)
Linoleic acid [*]	C ₁₈ H ₃₂ O ₂	0.39 (0.06)	1.73 (0.95)	0.37 (0.17)	2.69 (1.73)	1.57 (0.35)	1.41 (0.38)	1.70 (0.92)
Oleic acid [*]	C ₁₈ H ₃₄ O ₂	0.66 (0.43)	0.72 (0.25)	0.94 (0.58)	4.29 (2.38)	2.73 (1.77)	0.76 (0.40)	2.26 (1.25)
Stearic acid [*]	C ₁₈ H ₃₆ O ₂	2.56 (1.72)	1.57 (0.26)	1.14 (0.05)	2.87 (1.67)	3.04 (0.58)	2.56 (1.63)	2.53 (1.22)
Arachidic acid [*]	C ₂₀ H ₄₀ O ₂	0.00	0.00	0.06 (0.03)	0.12 (0.11)	0.00	0.25 (0.22)	0.00
Docosanoic acid [*]	C ₂₂ H ₄₄ O ₂	0.00	0.00	0.00	0.00	0.00		0.37 (0.19)
Tetracosanoic acid [*]	C ₂₄ H ₄₈ O ₂	0.00	0.00	0.00	0.00	0.00	0.00	1.81 (0.32)

Table 2. Continued

Compounds and extractives	Formula	1 year	2 year	3 year	4 year		5 year	
		Sapwood	Sapwood	Sapwood	Sapwood	Heartwood	Sapwood	Heartwood
Extractive		0.76 (0.13)	1.27 (0.10)	1.54 (0.52)	1.67 (0.37)	1.83 (0.56)	1.50 (0.55)	2.45 (0.16)
Sterols		3.25	3.09	3.40	7.36	16.20	9.51	28.20
Campesterol**	C ₂₈ H ₄₈ O	0.00	0.00	0.93 (0.37)	2.06 (0.93)	0.00	1.44 (0.74)	4.91 (2.24)
Stigmasterol**	C ₂₉ H ₄₈ O	0.00	0.86 (0.30)	0.49 (0.15)	0.00	2.02 (1.04)	0.58 (0.50)	2.71 (0.46)
β -sitosterol*	C ₂₉ H ₅₀ O	3.25 (1.60)	2.23 (0.80)	1.98 (1.09)	3.27 (1.86)	9.04 (0.69)	3.80 (0.19)	6.77 (0.81)
Urs-12-en-28-ol**	C ₃₀ H ₅₀ O	0.00	0.00	0.00	0.00	0.60 (0.31)	0.00	1.14 (0.29)
Cycloeucaleanol**	C ₃₀ H ₅₀ O	0.00	0.00	0.00	0.00	0.63 (0.44)	1.20 (1.04)	4.32 (1.08)
Cyclolaudenol**	C ₃₁ H ₅₂ O	0.00	0.00	0.00	2.03 (1.65)	3.30 (1.27)	2.49 (1.56)	6.61 (1.39)
Stigmast-5-en-3-ol, oleate**	C ₄₇ H ₈₂ O ₂	0.00	0.00	0.00	0.00	0.60 (0.18)	0.00	1.78 (0.41)
Other		3.22	0.00	0.41	0.00	3.86	0.00	4.23
1-Heneicosanol**	C ₂₁ H ₄₄ O	3.22 (2.44)	0.00	0.41 (0.17)	0.00	3.86 (1.41)	0.00	4.23 (1.38)

The SDs are given in the brackets.

Means of three trees, * compared with standard compound, ** compared with similarity index > 80% and/or with data from the literatures.

Data from NIST (2011).

Eucalyptus urograndis, *Eucalyptus pellita*, *Eucalyptus dunnii*, and *Eucalyptus grandis* × *Eucalyptus urophylla* clone wood, also reported the same compounds (Arisandi *et al.*, 2020; Silvério *et al.*, 2007). In *S. mahagoni*, palmitic, linoleic, oleic, and stearic acid compounds were the main fatty acid groups. Some studies also reported the same composition in other species, such as *E. globulus*, *E. pellita*, *E. grandis*, *E. dunnii* and *E. grandis* × *E. urophylla* clones (Arisandi *et al.*, 2020; Freire *et al.*, 2002b; Gominho *et al.*, 2020).

The sterol group was the second largest fraction after the formation of heartwood (Table 2, Fig. 4). In previous studies, sterols were mostly the second largest fraction as reported in *E. pellita*, *E. grandis*, *E. dunnii*, and *E. grandis* × *E. urophylla* clone (Arisandi *et al.*, 2020; Kilulya *et al.*, 2014). In *S. mahagoni*, the abundant sterol composition includes stigmasterol, β -

sitosterol, campesterol, and cyclolaudenol. Meanwhile, components of urs-12-en-28-ol, stigmast-5-en-3-ol, oleate cycloeucaleanol were the minor compounds. From these constituents, β -sitosterol was the largest compound in the sterol fraction. Similar results were also reported in previous studies such as on *E. globulus* clone, *E. pellita*, *E. grandis* × *E. urophylla* clone (Arisandi *et al.*, 2020; Freire *et al.*, 2002a, 2002b; Gominho *et al.*, 2020). According to Rastogi and Mehrota (1993), mahogany heartwood contains β -sitosterol, lupleol, cyclo swietenol, benzoic hedergenin, and cycloartenol.

3.2. Lipophilic composition in different tree age

Before heartwood formation (3 years), lipid groups of hydrocarbons increased from 1- to 2-year-old and then

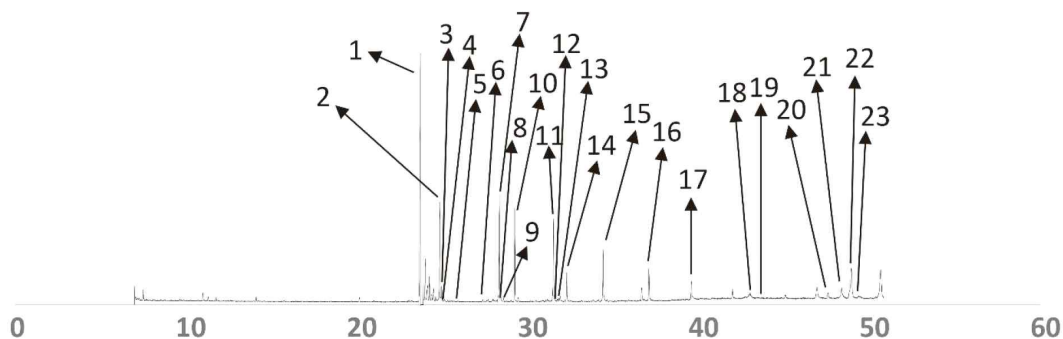


Fig. 3. GC-MS chromatogram of heartwood *n*-hexane extract (tree no. 2 of 4-year-old) from *Swietenia mahagoni* (TMCS derivatization method). Peak 1. internal standard: veratric acid (23.7 min), 2. cetene (24.8 min), 3. pentadecene (24.9 min), 4. undecane, 3-methylene- (25.1 min), 5. myristic acid (25.9 min), 6. pentadecanoic acid (27.6 min), 7. 1-octadecene (28.3 min), 8. hexadecene (28.4 min), 9. tridecane, 3-methylene- (28.5 min), 10. palmitic acid (29.2 min), 11. cyclotetracosane (31.5 min), 12. linoleic acid (31.8 min), 13. oleic acid (31.9 min), 14. stearic acid (32.3 min), 15. cycloeoicosane (34.4 min), 16. 1-heneicosanol (37.1 min), 17. cyclooctacosane (39.5 min), 18. urs-12-en-28-ol (43.6 min), 19. stigmast-5-en-3-ol, oleate (44.0 min), 20. stigmastrol (47.5 min), 21. cyclolaudenol (48.3 min), 22. β -sitosterol (48.9 min), and 23. cycloeucaenol (49.3 min). GC-MS: gas chromatography-mass spectrometry, TMCS: trimethylchlorosilane.

decreased drastically to 3-year-old namely 1-octadecene, cyclotetracosane, cycloeoicosane, and pentadecane (Table 2, Fig. 4). Meanwhile, the amount of hexadecane, heneicosane, and cyclooctacosane decreased from 1- to 3 years old. Furthermore, the number of fatty acids increased from 1- to 2-year-old and decreased to 3-year-old, specifically for palmitic and linoleic acids. The amount of oleic acid increased from 1 to 3 years old, and a reverse trend was found in the content of stearic acid. Dodecanoic, myristic, and pentadecanoic acid were only detected at the age of 1 year old. In addition, sterols content slightly increased from 1 to 3 years, even though the amount of β -sitosterol decreased. A similar trend was also shown in another compound known as 1-heneicosanol.

During heartwood formation (3 to 4 years), hydrocarbon components increased four times in the sapwood part. Components that contributed greatly to the increase in this number were pentadecane, and 1-octadecane. In addition, fatty acids and sterols increased twice. The fatty acid components that contribute to increasing levels

are linoleic, oleic, and stearic acids. Sterol components are β -sitosterol, campasterol, and cyclolaudenol. The opposite pattern was found in the 1-heneicosanol.

After heartwood formation at 4-year-old, the level of hydrocarbon and fatty acid contents decreased almost twice times in the sapwood (Table 2, Fig. 4). This was because the components of pentadecane, hexadecane, heptadecane, and heneicosane in the sapwood decreased dramatically at the age of 5 years for hydrocarbons fractions, while the fatty acid groups contained palmitic, linoleic, and oleic acid. In the heartwood, generally all fractions increased (except for the hydrocarbon fraction). The composition of sterol fraction almost doubled from 16.2% to 28.2%. After the formation of heartwood, the largest fraction was hydrocarbons, followed by sterols and fatty acids. These results are similar to a study conducted by Silvério *et al.* (2007) and Gutiérrez *et al.* (1999) on *E. urograndis* clones and *E. globulus* respectively. The results showed that hydrocarbons, fatty acids, and sterols were the main lipid groups identified.

Decreasing or increasing in some extractive com-

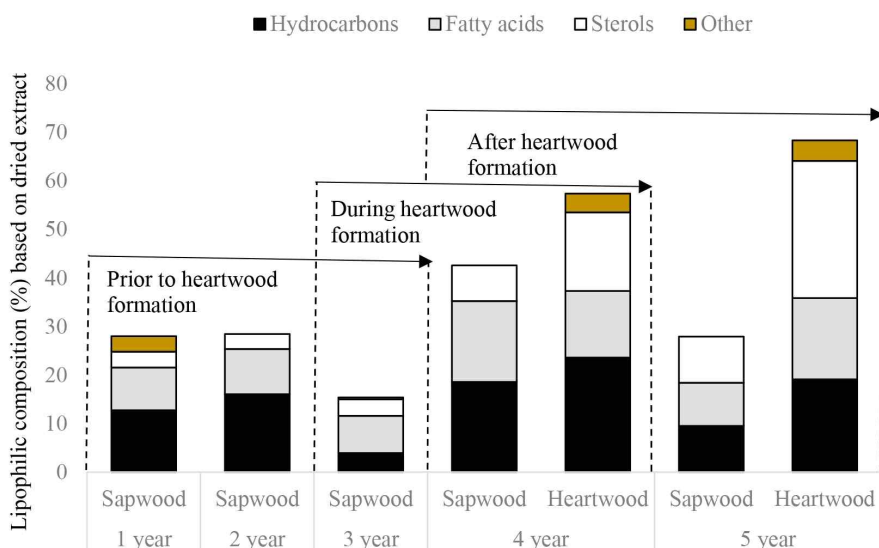


Fig. 4. Lipophilic extractive composition (%) based on dried *n*-hexane extract from *Swietenia mahagoni* wood.

pounds due to the age of the tree is influenced by several factors. The extractive content increases, it is thought to be because the cambium vessels in older trees are more likely to divide and multiply into parenchyma cells, so that the formation of parenchyma cells increases. Therefore, the extractive content tends to increase (Hamidah *et al.*, 2009). The similar pattern had been reported in previous studies in *E. globulus*, *E. grandis*, *E. urophylla*, and *Tectona grandis* (Lestari *et al.*, 2016; Lukmandaru, 2009; Miranda and Pereira, 2002; Santana *et al.*, 2012). The levels of extracts or compounds drop, this may be because the compounds are converted to other secondary compounds for heartwood formation or other physiological processes, such as respiration, photosynthesis, etc.

3.3. Lipophilic composition in radial profiles

3.3.1. Distribution of fatty acids

The fatty acid composition of the heartwood differed

from that of the sapwood (Table 2, Fig. 4). The sapwood was rich in fatty acids with 16.70%, while the heartwood part was 13.70% at 4 years of the tree. Even though, the compound of palmitic acid represented 6.08% in the sapwood, while in the heartwood it was only 6.27%. Fatty acid composition tended to decrease from sapwood to heartwood, including unsaturated fatty acids (oleic and linoleic). Saranpää and Nyberg (1987) reported that the amount of oleic (18:1) and linoleic (18:2) acids in the triacylglycerol fraction decreased towards the heartwood. This suggests a general unsaturation of the triacylglycerols during heartwood formation. A similar pattern was also reported by Bertaud and Holmbom (2004) and Ekman (1979), where the amount of lipophilic extractives was greater in sapwood than in heartwood of *Picea abies*. This may be due to the greater number of triglycerides. Triglycerides and sterile esters are present in living parenchyma cells. As heartwood forms and parenchyma cells die, triglycerides are hydrolyzed by lipase enzymes. However, triglycerides were not quantified in this study and further research is

needed in this case. Hemingway and Hillis (1971) also found that the metabolized glyceride esters were rich in oleic acid (18:1), whereas linoleic acid (18:2) and saturated fatty acids were concentrated in other esters, such as wax in *P. radiata*. Another possibility is that at 4 years, heartwood was just beginning to form, so that the lipids in the sapwood have not all been fully metabolized into heartwood extractives such as fatty acids. Magel *et al.* (1991, 1994) reported analyses of heartwood extractives on individual tree rings from the cambium to the pith. They found that the physiological process of heartwood formation starts on the third or fourth growth and was completed after 2 years. Dünisch *et al.* (2010) also stated that the extractive content of heartwood was higher in the mature heartwood (9.80%–10.30%) than in the heartwood formed during the juvenile phase. Hillinger *et al.* (1996) and Magel *et al.* (1991) mentioned that carbohydrates and lipids were soluble in methanol in the sapwood part of black locust. The living tree uses these substances especially during the process of heartwood formation.

There were marked differences in the fatty acid composition of sapwood and heartwood between 4- and 5-year-old trees (Table 2, Fig. 4). At 5 years of age, almost all fatty acid compounds, including unsaturated fatty acids (oleic and linoleic) increased from sapwood to heartwood. Heartwood contained 16.80% of fatty acid content while sapwood contained 8.89%. Similar trends have been reported by some previous studies in several other species such as *E. globulus* (Gominho *et al.*, 2020), *Eucalyptus camaldulensis* (Benouadah *et al.*, 2017), *Acacia* hybrid (Soon and Chiang, 2012), *Pinus sylvestris* (Saranpää and Nyberg, 1987) and *P. radiata* (Hemingway and Hillis, 1971). It is clear, therefore, that by the age of 5 years, most of the lipids have been converted to extractive compounds in the heartwood. Since ray parenchyma cells have starch-containing plastids (amyloplasts; Parameswaran and Bauch, 1975), it is reasonable to assume that the fatty acids and lipids found in wood are

at least partly synthesized in living wood cells and not transported from the photosynthetic cells of the tree. Plastids are thought to be the sole source of fatty acids in plants (Stumpf, 1980). However, the fatty acids present in wood do not always appear to be identical to those present in leaves, and their relative amounts may be very different.

Other fatty acids such as dodecanoic, myristic and arachidic acids were only found in sapwood. In contrast, long chain fatty acids such as docosanoic and tetracosanoic acids were only found in the heartwood. Arisandi *et al.* (2020) also reported that long chain fatty acids such as docosanoic and tetracosanoic acids were more abundant in the heartwood than in the sapwood of *E. pellita* species. The concentration of fatty acids in heartwood samples, especially long-chain fatty acids suggests that these compounds are produced extensively during heartwood formation as a defence mechanism against microorganisms and insects or fungal decay (Blanchette and Biggs, 1992; Metsä-Kortelainen and Viitanen, 2009). On the other hand, fatty acids appear to have antibacterial activity (Desbois and Smith, 2010). Fatty acids can act as ionic surfactants and have antibacterial and antifungal properties at low pH (Hayes and Berkovitz, 1979). Palmitic, stearic, oleic, linoleic and linolenic acids are well known to have antibacterial antioxidant activities (Karimi *et al.*, 2015; McGaw *et al.*, 2002).

3.3.2. Distribution of hydrocarbons

The composition of hydrocarbons in sapwood differed from that in heartwood. In general, the content of hydrocarbons (including major compounds) was higher in heartwood than in sapwood at both 4 and 5 years of age. Hydrocarbon composition in the sapwood was 18.60% and 9.51%, whereas in the heartwood it was 23.60% and 19.10% at 4 and 5 years of age, respectively (Table 2). Hydrocarbons like fatty acids and sterols are lipophilic compounds. Hydrocarbons are commonly found in wood, for example in eucalyptus wood. del Río *et al.*

(1998) reported that in *E. globulus* wood extractives consist mainly of hydrocarbons, fatty acids, waxes, steroids and triglycerides. There is very little discussion on the hydrocarbon composition of sapwood and heartwood (Gominho *et al.*, 2020), except for the general hydrocarbon composition of wood, bark and pulp (del Río *et al.*, 1998; Gominho *et al.*, 2020; Gutiérrez *et al.*, 1999, 2006; Kilulya *et al.*, 2012, 2014). The function of hydrocarbons in relation to the heartwood formation process may be similar to that of fatty acids and sterols as they are still in the same lipid class. Previous research indicated that four hydrocarbon compounds, including octacosane, tetracosane, hentriacontane and heneicosane, act as antifungal agents on *Cryptococcus neoformans* and *Phanerochaete chrysosporium* (Masendra *et al.*, 2015; Passos *et al.*, 2003). However, the more specific role of hydrocarbons in the process of heartwood formation needs to be further investigated.

3.3.3. Distribution of sterols

The highest number of sterols was found in the heartwood (Table 2, Fig. 4). The composition of the sterol fraction in sapwood at 4 and 5 years of age was 7.36% and 9.51%, respectively. Meanwhile, in heartwood was 16.20% and 28.20%, respectively. It is interesting to note that the heartwood, which was mostly dead, contained the highest concentration of sterols (Fig. 4). The main sterols detected were stigmaterol, β -sitosterol, campesterol and cyclolaudenol of which β -sitosterol was the largest compound in the sterol fraction with a composition of 3.27% to 9.04%. In addition, compounds such as urs-12-en-28-ol and stigmast-5-en-3-ol, oleate, cycloeucaenol (4 years) were only detected in the heartwood. The increase in sterol compounds from the sapwood to the heartwood region is similar to that reported in previous studies in several species, such as *E. globulus* (Gominho *et al.*, 2020), *E. camaldulensis* and *Pinus halepensis* (Benouadah *et al.*, 2017), and *Acacia* hybrid (Soon and Chiang, 2012). This increase in free

sterols in heartwood may be due to their involvement in self-defence mechanisms (Hillinger *et al.*, 1996). Höll and Goller (1982) also found the highest concentrations of free sterols and sterol esters in the inner heartwood of spruce and suggested that these compounds are synthesized when the tree is young. Höll and Lipp (1987) suggested that sterols accumulate in the heartwood of *Pinus sylvestris* during the early stages of the life cycle. Another possibility is the amount of sterol ester compounds hydrolyzed during the heartwood formation, as occurs in *P. abies* (Bertaud and Holmbom, 2004).

It can be said that different developmental stages of parenchyma cells have certain characteristics in their lipid metabolism. In observations on the species *Robinia pseudoacacia*, Kampe and Magel (2013) explained that the amount of phospholipids and parenchyma cell integrity parameters decreased towards the transition zone. Only trace amounts were found in the heartwood. The amount of free sterols decreased from sapwood to the transition zone, but reached a maximum in the heartwood. According to the researchers, the first stage of cell damage may be due to a decrease in both compounds. The increase in free sterols in heartwood may also be due to a possible involvement in defence mechanisms against pathogens (Hillinger *et al.*, 1996). For example, the compound of β -sitosterol is known to have antifungal activity against *Fusarium* spp. (Kiprono *et al.*, 2000) and inhibits the growth rate of the fungus *P. chrysosporium* (Masendra *et al.*, 2020). Steroid constituents such as cycloeucaenyl acetate and stigmasta-3,5-diene from *n*-hexane extract of *E. pellita* have been specifically associated with increased toxicity to *Cryptotermes termites* (Masendra *et al.*, 2021). A high accumulation of steroid compounds was also believed to affect potent antioxidant activity (Masendra *et al.*, 2021). Furthermore, phytosteroids may be useful in treating inflammatory diseases, such as inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, asthma, and cardiovascular disease (Cheng *et al.*, 2022).

The depletion of reserve substances, starch, NSC and triacylglycerol has previously been recognized as a marker of parenchyma cell disintegration (Kampe and Magel, 2013). The abundant starch granules in the outer sapwood gradually decrease from the middle sapwood towards the transition zone. The starch contained in the middle sapwood is hydrolyzed by phosphatases into sugar phosphates, thus becoming a precursor for lipid or extractive synthesis. The researchers also found lipophilic spots in the axial parenchyma and spokes in the outer sapwood. These spots increased significantly in size in the middle and inner sapwood cells and in the transition zone. Within these tissues, lipids and carbohydrates are thought to provide the carbon skeleton for the synthesis of heartwood extractives. Thus, lipids and starch are degraded during heartwood formation and play an important role in providing the carbon skeleton for the biosynthesis of phenolic extractives and thus the ageing of ray parenchyma cells (Hillis, 1987; Lamloom and Savidge, 2006; Magel, 2000, Song *et al.*, 2011). This is consistent as reported by Arisandi *et al.* (2022), where the main soluble sugar compounds, monosaccharides (fructose, glucose, mannose and xylose) and disaccharides (sucrose) decreased from sapwood to heartwood. Even the compounds of arabinose, galactose and sucrose at 5 years old were found only in sapwood in *S. mahagoni*.

4. CONCLUSIONS

In conclusion, the present study was the first report on the lipophilic components found in the sapwood and heartwood of *S. mahagoni*, specifically regarding their relevance to heartwood formation. The *n*-hexane extract content varied between 0.76% and 2.45%, based on the weight of dried wood. Furthermore, four lipophilic fractions including hydrocarbons, fatty acids, sterols, and other compound (1-heneicosanol) were identified in *S. mahagoni* wood. The major groups of constituents

consisted of sterols (β -sitosterol, stigmasterol, campesterol, and cyclolaudenol), fatty acids (palmitic, oleic, linoleic, and stearic acids), and hydrocarbons (pentadecane, 1-octadecane, hexadecane, cyclotetracosane, cycloicosane, and cyclooctacosane), while 1-heneicosanol was minor compound. After heartwood formation, the hydrocarbon fraction was the largest followed by sterols, fatty acids, and 1-heneicosanol. Lipophilic extractives increased with tree age, except for the hydrocarbon fraction which decreased at 3 and 5 years (sapwood). Furthermore, the distribution of lipophilic extractives increased drastically from sapwood to heartwood, especially at 5 years, except for fatty acids at 4 years, which showed the opposite trend. It is suggested that at the age of 4 years, lipids were not fully converted to heartwood extractives at 4 years of age, when heartwood was just being formed. In addition, the role of hydrocarbons in relation to heartwood formation needs to be further investigated.

CONFLICT of INTEREST

No potential conflict of interest relevant to this article was reported.

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